Comparative molecular characterization of *Corynebacterium pseudotuberculosis* strains isolated from Hungarian sheep and goat flocks

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The genus Corynebacterium belongs to the order Actinomycetales that also includes the genera Mycobacterium, Nocardia, Streptomyces. Although this group is very heterogeneous, most of the species share particular characteristics, such as the cell wall composition and the high G+C ratio of the genomes. *C. pseudotuberculosis* is a Gram-positive, club-shaped rod, facultative intracellular bacteria that is commonly found in the environment as well as on the skin and mucous surfaces of animals. Based on biochemical features, the strains are classified into two biovars (ovis and equi). The strains within the biovar ovis presented negative nitrate reduction and mainly affect sheep and goats, causing caseous lymphadenitis (CL). The strains biovar equi reduce nitrate and cause ulcerative lymphangitis of the distal extremities, ventral abscesses of the thorax and abdomen, and furunculosis in horses. Occasionally, both types could generate subcutaneous abscesses or visceral organs in cattle. In addition, in rare instances, other domestic or wild animal species, and even human are affected.

Due to the sale restrictions of pedigree sires, the reduction of milk, meat and woollen yields, expressive losses in reproductive efficiency, and condemnation of carcasses in slaughterhouse the caseous lymphadenitis causes significant economic losses to sheep producers worldwide. While the illness is common on the tropical areas, it occurs only sporadically in the Mediterraneum. In default of the appropriate import regulations it has spread throughout the world by the pedigree sires.

The aim of our study was to molecular comparison of phenotypically characterized *C. pseudotuberculosis* strains have been collected form sheep and goat flocks in different areas of the country over the past twenty years (1994-2014). We opted for the multilocus sequence typing (MLST) analysis. In the absence of data from literature, we were going to use the MLST had been developed for the analysis of human pathogenic *C. diphtheriae* strains recently (2010). However the applied primers were *C. diphtheriae* specific, the developing of own system was needed. For designing of appropriate primers, we have collected and compare of sequences of 16 commonly applied housekeeping using whole genomes of *C. pseudotuberculosis* at the Genebank. During the examination of the individual genes, the *C. pseudotuberculosis* and *C. diphtheriae* strains proved to be distinct in any case and two biovars were found to various in some measure. Finally, the 600-800 bp long regions covering utmost single-nucleotide polymorphism (SNP) between the biovars and present identical phylogenetic topology of the entire genes of *dna*K, *odh*A, the *gro*L1 and *inf*B were examined in detail.

Although each of our strains were classified to *C. pseudotuberculosis* biovar ovis, the MLST examination demonstrated that the two biovars are separated at the level of the individual housekeeping genes. In light of the results is thought-provoking that the strains belong to ovis and the equi biovars represent the same taxonomic group despite the differences of genetic and phenotypic properties (nitrate reduction), of host spectrum (sheep, goat; horse) and of the manifestation of induced disease.

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